REMARKS

Claims 1-7, 9-14, 19-21, 15-18 and 23 are now in the application. Claim 15 has been amended to recite "melting point" in place of "melting points" for purposes of clarification and not to limit its scope. Claim 24 has been cancelled without prejudice or disclaimer. The amendments do not introduce any new matter. Claims 15-18 and 23 are drawn to the elected invention. Claims 1-7, 9-14 and 19-21 are drawn to non-elected invention and may be cancelled by the examiner upon the allowance of the claims directed to the elected invention.

The rejections under 35 USC 112, second paragraph have been overcome by the above amendments.

Before addressing the rejections of the claims over the cited art, a discussion of the present invention would be helpful. In particular, the present invention is directed to a process for producing a ubiquinone supplementation food which comprises dissolving ubiquinone in an oil/fat under heating at a heating temperature of not lower than the melting point of ubiquinone, and adding the obtained mixture to a food material.

Ubiquinone is a substance indispensable for maintaining biological functions, but the ubiquinone content in the body decreases markedly due to aging and various stresses to which the living body is subjected. In order to supplement ubiquinone, people usually take a supplement in the form of tablets or capsules. In this case it is necessary to purposely take a supplement apart from ordinary foods.

It is also possible to supplement a sufficient amount of ubiquinone from ordinary foods. It enables the taking of ubiquinone more easily and conveniently. However, it is necessary to add a large amount of ubiquinone to food materials in the preparation process of foods since the ubiquinone content in ordinary foods is very low. In this case there arise serious problems of separation or localization of ubiquinone, which adversely affects flavor, texture and appearance of the foods. If ubiquinone localizes in the food, for example, the food partially shows yellow color which is the color of ubiquinone. The problems are inherent to ordinary foods, but there is

no such problem in the tablets or capsules. In case of capsules or tablets, it is only necessary to simply encapsulate a large amount of ubiquinone in capsules or tablets since uniformity (with regard to the appearance and flavor) is not a critical issue.

The present invention is concerned with solving the problems of separation or localization and makes it possible to provide a ubiquinone supplementation food excellent in flavor, texture and appearance. The ubiquinone supplementation food can be produced by adding a composition containing ubiquinone and an oil/fat to a food material in the manufacturing process. However, the solubility of ubiquinone in oils/fats is remarkably low at room temperature (please see page 6, lines 3 to 10 of the specification). In order to add a large amount of ubiquinone to an oil/fat, ubiquinone is completely dissolved in an oil/fat under heating at a heating temperature of not lower than the melting point of ubiquinone. This process enables the addition of ubiquinone over its solubility limit in the oil/fat.

Claims 15-16, 18 and 23-24 were rejected under 35 USC §103 over US Patent 6,616,942 to Udel in view of US Patent 4,049,831 to Ono. The cited references do not render obvious the present invention.

Udel suggests a method of producing a soft gel capsule comprising heating rice bran oil to 50 to 60°C, adding bee's wax and mixing them together to obtain a uniform mixture, cooling the mixture to 35 to 45°C, adding coenzyme Q_{10}, MCT (medium-chain triglycerides) and other components and mixing together, cooling the resultant mixture to 25 to 30°C and encapsulating it in a soft gel capsule (please see col. 3, lines 7 to 45).

As appreciated by the Examiner, Udel fails to teach dissolving ubiquinone in an oil/fat under heating at a temperature of not lower than the melting point (about 48°C) of ubiquinone. According to the Office Action, it would have been obvious to add ubiquinone as a heated liquid above its melting point in order to provide a uniformly distributed ubiquinone product. However, according to Udel, rice bran oil is once heated to 50 to 60°C and bee's wax is added, but the mixture is purposely cooled to 35 to 45°C and then ubiquinone is added with other components. Therefore, there is no motivation to add ubiquinone under heating at a temperature

of not lower than the melting point (about 48°C) of ubiquinone. To do so would be contrary to the suggestions of Udel.

First of all, Udel relates to a production of soft gel capsule formulation. Soft gel capsules are distinct from ordinary foods according to the present invention. Therefore, the suggestions of Udel do not belong to the field of foods and those skilled in the art would not recognize the problem of separation or localization of ubiquinone in foods. As discussed above, the problem addressed by the present invention is not a problem of concern with soft gel capsules.

Further, according to Udel, bee's wax is used as a suspension agent for subsequent ingredients (col. 3, lines 12 to 13) which increases viscosity to keep insoluble components from settling to one side of the soft gel capsule (col. 3, lines 36 to 38). It is not necessary to retain ubiquinone in a uniformly dissolved and dispersed state in soft gel capsules. Accordingly, the present invention (claim 15) is not at all obvious from Udel.

Regarding claim 16, it is important to note that MCT used in Udel is not a solid fat.

First of all, it is known that MCT is obtained by fractionation of low melting point fraction, namely liquid oil, of coconut oil. The attached reference (Ferencikova et al., Physiol. Res. 52: 73-38, 2003; hereinafter Reference 1) teaches the composition of Miglyol 812 used in Udel (page 74, lines 11 to 14). According to Reference 1, Miglyol 812 is a mixture of triglycerides containing C_6-C_{12} fatty acids as the constituent fatty acids. Specifically, C_8 fatty acid (caprylic acid) and C_{10} fatty acid (capric acid) account for more than 80% of the fatty acids which constitute the triglycerides while lauric acid accounts for 5% at most. Accordingly, those skilled in the art would not believe that melting point of MCT is 20°C or higher.

Ono uses hydrogenated coconut oil having a melting point of 38°C. According to the Office Action, the melting point of triglycerides can be higher or lower depending on the degree of hydrogenation (please see page 5, first paragraph). However, the Examiner's attention is kindly directed to the attached reference (Akpan et al. Pakistan Journal of Nutrition 5 (2): 106-109, 2006; hereinafter Reference 2). Reference 2 discloses the fatty acid profile of various

coconuts (Table 2). Medium chain fatty acids constituting coconut oils are caproic acid, caprylic acid, capric acid and lauric acid, which are all saturated fatty acids. Therefore the MCT fraction of coconut oil is not hydrogenated and the melting point of MCT does not change by hydrogenation.

According to Table 2 of Reference 2, coconut oils are composed of C_6-C_{20} fatty acids, lauric acid accounting for about 38-45% and C_6-C_{12} fatty acids accounting for about 60% in total. Meanwhile, fatty acids other than medium chain fatty acids, i.e. myristic acid, palmitic acid, stearic acid, arachidonic acid, oleic acid and linoleic acid, account for about 40%. The melting point of coconut oil increases by hydrogenation because C=C bonds of unsaturated long chain fatty acids such as arachidonic acid, oleic acid and linoleic acid are hydrogenated, so that arachidic acid and stearic acid and the like are produced. This is common knowledge to those skilled in the art.

As discussed above it is inappropriate to interpret the teaching of Ono as a teaching that the melting point of MCT increases by hydrogenation. Accordingly, those skilled in the art would not be motivated from the teachings of Udel and Ono to use a solid fat. The attached references are being presented in this response to address comments made in the Final Office Action and therefore were not presented earlier.

Claims 15-18 and 23-24 were rejected under 35 USC §103 as US patent application publication 2003/0113307 to Selzer in view of Udel and Ono. The cited references do not render obvious the present invention. The above discussions of Udel and Ono are incorporated herein by reference.

As appreciated by the Examiner, Selzer fails to teach dissolving ubiquinone in an oil/fat under heating at a temperature of not lower than the melting point (about 48°C) of ubiquinone. Selzer merely suggests mixing an oil phase containing coenzyme Q_{10} and oil.

Further, Selzer suggests that coenzyme Q_{10} is not very stable and deteriorates at temperatures above 115°F, namely 46°C (paragraph [0006]). Accordingly, Selzer teaches away

from dissolving ubiquinone in an oil/fat under heating at a heating temperature of not lower than the melting point of ubiquinone.

First of all, the composition according to Selzer is used as a dietary supplement and/or therapeutic supplement. The suggestions of Selzer do not belong to the field of ordinary foods according to the present invention. Therefore, those skilled in the art would not recognize from the teachings of Selzer for solving the problem of separation or localization of ubiquinone in foods.

According to the present invention, on the other hand, ubiquinone is dissolved in an oil/fat under heating at a temperature of not lower than the melting point (about 48°C) of ubiquinone. Ubiquinone is dissolved purposely at high temperatures in order to prevent separation or localization of ubiquinone in ordinary foods.

As discussed above, neither Udel nor Ono suggest dissolving ubiquinone in an oil/fat under heating at a temperature of not lower than the melting point (about 48°C) of ubiquinone. Selzer does not remedy the deficiencies of Udel and Ono. Accordingly, the present invention is not rendered obvious even if these references are combined.

Therefore, to modify the cited references by heating at a temperature of not lower than the melting point (about 48°C) of ubiquinone would be contrary to their disclosures. It is improper to disregard teachings that lead away from the invention in evaluating non-obviousness. All of the teachings in the art must be considered including those that teach away. Please see *In re Mercier* 185 USPQ 774 (CCPA, 1975). Moreover, where, as here, the teachings of the prior art would discourage persons skilled in the art from doing what applicant teaches and claims, the art establishes the "very antithesis of obviousness". Please see, *In re Rosenberger*, 156 USPQ 24 (CCPA, 1967) and *In re Buehler*, 185 USPQ 781 (CCPA, 1975).

In view of the above, consideration and allowance are respectfully solicited.

In the event the Examiner believes an interview might serve in any way to advance the prosecution of this application, the undersigned is available at the telephone number noted below.

The Office is authorized to charge any necessary fees to Deposit Account No. 22-0185, under Order No. 21581-00488-US from which the undersigned is authorized to draw.

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Hepatotoxic Effect of D-Galactosamine and Protective Role of Lipid Emulsion

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Summary

D-galactosamine is a hepatotoxic agent, which induces diffuse injury of liver tissue followed by the regeneration process. Our data showed a high increase of serum aminotransferases after D-galactosamine administration, which indicates a high extent of liver injury. When lipid emulsion was applied immediately after D-galactosamine, the increase of serum aminotransferases was greatly reduced. In addition, the decrease of the cytochrome c oxidase activity induced by D-galactosamine was not observed after lipid emulsion administration and the increase of total liver oxidative capacity in the regeneration period due to activated mitochondrial biogenesis was accelerated. All these findings indicate a protective effect of lipid emulsion administration against D-galactosamine toxicity.

Key words

D-galactosamine hepatotoxicity • Liver regeneration • Cytochrome c oxidase • Lipid emulsion

Introduction

Liver regeneration is an example of tissue recovery after injury. This proliferation process can be induced in experimental conditions by partial hepatectomy or by various hepatotoxic chemical agents (tetrachlormethane, D-galactosamine, thioacetamide).

D-galactosamine (GalN) is known for inducing the features of acute hepatitis in rats. The toxic effect of GalN is connected with an insufficiency of UDP-glucose and UDP-galactose and the loss of intracellular calcium homeostasis. These changes affect cell membranes and organelles and the synthesis of proteins and nucleic acids (Keppler and Decker 1969). After GalN application, the location of proteoglycans is changed in the rat liver (Sasaki *et al.* 1996). GalN also inhibits the energy metabolism of hepatocytes (Mangeney-Andreani *et al.* 1982). This and a further study (Sire *et al.* 1983) show that GalN injures the enzymes involved in the transport of substrates to the mitochondria and modifies the phospholipid composition of membranes. The activity of mitochondrial enzymes is dependent on lipoprotein interactions and their modification by GalN may indirectly affect enzymes activities.

Damage of liver structure and function induces the regenerating process. An important role in this regeneration process is played by the cytokine IL-6. It prevents the progression of liver necrosis and thus enhances the survival of intoxicated animals and is also involved in initiating liver regeneration (Galun *et al.* 2000, Hecht *et al.* 2001).

After GalN injury, liver responds by activation of progenitor cells that proliferate and then differentiate into mature hepatocytes. Adult hepatocytes can also

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proliferate after GalN injury but these hepatocytes do not undergo dedifferentiation (Dabeva and Shafritz 1993). Other findings have demonstrated that hepatocytes can divide to restore the liver mass after GalN liver injury (Kitten and Ferry 1998).

In our study, we tested the effect of GalN administration on various parameters of liver metabolism and especially on cell energy metabolism. We also studied the effect of lipid emulsion on hepatotoxic injury because lipids are a preferred source of energy in regenerating liver tissue. We continued our previous studies (Červinková *et al.* 1995, Červinková and Drahota 1998) which showed that after partial hepatectomy and thioacetamide-induced liver injury the administration of lipid emulsion rich in medium chain fatty acids significantly attenuates the necrotic process induced by thioacetamide and accelerates the process of liver regeneration.

Methods

The experiments were made on male Wistar rats, with initial weight of 180-215 g. The rats were housed at 23±1°C, 55±10 % relative humidity, air exchange 12-14 times/h, and at 12-hour light-dark cycle periods (6:00 to 18:00). The animals had free access to standard laboratory rat chow (DOS 2B, Velaz, Prague, CR) and tap water. The animals received care according to the guidelines set by the institutional Animal Use and Care Committee of the Charles University.

D(+)-Galactosamine (Sigma) was administered i.p. in a single dose of 800 mg/kg of body weight. Lipid emulsion (Lipofundin MCT/LCT 10 % / Miglyol[812], in a ratio 1:1 Lipofundin / Miglyol) was given by stomach tube in a 15 ml/kg dosage twice a day (in 6-h intervals between both dosages) immediately after GalN administration and continued until the end of the experiment. Lipofundin MCT/LCT 10 % (B. Braun, Melsungen, Germany) is a sterile, non-pyrogenic fat emulsion for intravenous administration with following composition: soybean oil 50 g, medium-chain triacylglycerols (a mixture of neutral TAG of mainly caprylic acid 60 % and capric acid 40 %) 50 g, egg yolk phospholipids 12 g, glycerol 25 g, water for injections to 1000 ml. Miglyol[812] (Dynamit Nobel, Germany) is a neutral oil containing caproic acid (max. 3 %), caprylic acid (50-65 %), capric acid (30-45 %) and lauric acid (max. 5 %). Control animals received a saline solution instead of the lipid emulsion in the same manner. Rats were sacrificed 24, 48, or 72 h after GalN application by exsanguination from the abdominal aorta.

The liver tissue was homogenized in 0.25 M sucrose, 10 mM Tris-HCl, 1 mM EDTA medium, pH 7.4 and mitochondria were isolated according to the previously described method (Schneider and Hogeboom 1950) and cytochrome c oxidase activity was evaluated spectrophotometrically using reduced cytochrome c (Kalous *et al.* 1989). The activity of enzyme was expressed as quantity of cytochrome c oxidized per minute per mg of protein (μmol cytochrome c/min/mg protein). Total cytochrome c oxidase activity was calculated per organ on the basis of specific activity per mg protein and total protein in the whole liver.

Proteins were determined according to Lowry *et al.* (1951) using bovine serum albumin as a standard. Liver protein was expressed as mg per g of wet weight or as g of total protein in the whole organ on the basis of total liver wet weight.

Table 1. Changes of serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activity after D-galactosamine injury.

Time after GalN (h)	ALT (μkat/l)			AST (μkat/l)		
	Control	GalN	GalN + Lipid emulsion	Control	GalN	GalN + Lipid emulsion
0	0.7±0.16	–	–	1.4±0.16	–	–
24	–	10.8±4.2	7.2±3.2	-	7.5±3.5	7.3±5.1
48	–	19.5±12	2.7±1.1*	-	15.0±10	3.1±0.7*
72	–	1.6±0.7	0.8±0.2*	–	2.2±0.7	1.4±0.2*

*$p<0.05$, ** $p<0.01$ indicate significant differences between groups with and without lipid emulsion.*

The activity of aspartate aminotransferase and alanine aminotransferase in the serum was determined using a Sigma kit (Bergmayer *et al.* 1978).

The values were expressed as means ± S.D. Each group consisted of 6 animals. The statistical differences between individual groups were calculated by Student's t-test.

Table 2. Changes of liver protein after D-galactosamine injury

Time after GalN (h)	Liver protein (mg/g wet weight)			Total liver protein (g)		
	Control	GalN	GalN + Lipid emulsion	Control	GalN	GalN + Lipid emulsion
0	325.0±26.2	–	–	2.2±0.2	–	–
24	–	$254.2\pm29.4^{++}$	270.8±14.4	–	$1.78\pm0.10^{++}$	2.06±0.18*
48	–	$231.0\pm17.2^{+++}$	245.1±9.2	–	$1.62\pm0.23^{++}$	2.00±0.16**
72	–	$193.0\pm9.8^{+++}$	205.6±27.1	–	$1.76\pm0.17^{++}$	1.67±0.22

*$^{++}$ $p<0.01$, $^{+++}$ $p<0.001$ indicate significant differences between control and GalN group; * $p<0.05$, ** $p<0.01$ indicate significant differences between groups with and without lipid emulsion.*

Table 3. Content of liver triacylglycerols after D-galactosamine injury

Time after GalN (h)	Liver triacylglycerols (mg/g liver tissue)		
	Control	GalN	GalN + Lipid emulsion
0	4.81±1.07	–	–
24	–	$7.18\pm1.58^{+}$	29.73±7.46***
48	–	$8.37\pm2.30^{+}$	35.35±6.96***
72	–	$6.75\pm0.91^{++}$	30.44±7.99***

*$^{+}p<0.05$, $^{++}p<0.01$ indicate significant differences between control and GalN group; *** indicate significant difference ($p<0.001$) between groups with and without lipid emulsion.*

Results

In our experiments, we assessed the extent of liver injury 24, 48, and 72 h after D-galactosamine administration by the increase of serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities (Table 1). We found a high increase of ALT and AST activities 24 and 48 h after GalN administration. The activities of both enzymes were normalized after 72 h, which indicated an activation of the regeneration process. The increase of both aminotransferases was significantly lower when the lipid emulsion was applied after GalN and also the recovery of normal aminotransferase activities in serum was faster.

Changes of total liver protein (Table 2) showed a significant decrease during the whole period studied. The administration of lipid emulsion reduced the decrease of total protein at 24-h and 48-h intervals. At 72-h interval, no difference between GalN and GalN plus lipid emulsion groups was found. Values of liver protein expressed per g wet weight showed a significant decrease after GalN at all tested intervals; however, the protective effect of lipid emulsion was not observed (Table 2). This could be explained by an interference of increased triglyceride content in the intoxicated liver (Table 3).

We also tested changes of liver oxidative capacity after GalN administration according to the activity of liver cytochrome c oxidase. As demonstrated in Table 4, total liver oxidase capacity was decreased 24 h after galactosamine administration. The total cytochrome c oxidase activity was again recovered 48 h after GalN administration and it was significantly higher

at 72 h compared with the control group. Data in Table 4 indicate the activation of cell oxidative capacity during the regeneration process induced by GalN intoxication. This was also confirmed by data on specific cytochrome c oxidase activity in the homogenate, which indicated a significant increase of this enzyme activity at 48-h and 72-h intervals after GalN administration. Because specific cytochrome c oxidase activity of isolated mitochondria was not changed (Table 5), the increase of homogenate activity at 72 h after GalN administration indicates that the amount of mitochondrial protein is increased.

Application of lipid emulsion had the most pronounced effect on both total content and activity of cytochrome c oxidase 24 h after GalN administration, when the lipid emulsion completely prevented the decrease of enzyme activity induced by D-galactosamine. The protective effect of lipids was evident at 48-h and 72-h intervals after GalN administration, however, it was less pronounced in comparison with the 24-h interval (Table 4).

Table 4. Changes of cytochrome c oxidase in liver homogenate after D-galactosamine injury

Time after GalN (h)	Total content of cytochrome c oxidase (mmol cytochrome c/min/liver)			Activity of cytochrome c oxidase (μmol cytochrome c/min/mg protein)		
	Control	GalN	GalN + Lipid emulsion	Control	GalN	GalN + Lipid emulsion
0	0.93±0.10	–	–	0.43±0.07	–	–
24	–	$0.70\pm0.13^{++}$	$1.01\pm0.09^{***}$	–	0.39±0.07	$0.49\pm0.03^{*}$
48	–	0.95±0.18	$1.17\pm0.09^{*}$	–	$0.58\pm0.04^{++}$	0.59±0.03
72	–	$1.25\pm0.18^{++}$	$1.46\pm0.12^{*}$	–	$0.71\pm0.10^{+++}$	$0.88\pm0.14^{*}$

$^{++}p<0.01$, $^{+++}p<0.001$ indicate significant differences between control and GalN group.; $^{*}p<0.05$, $^{***}p<0.001$ indicate significant differences between GalN group and group with and without lipid emulsion.

Table 5. Activity of mitochondrial cytochrome c oxidase after D-galactosamine injury

Time after GalN (h)	Cytochrome c oxidase activity (μmol cytochrome c/min/mg mitochondrial protein)		
	Control	GalN	GalN + Lipid emulsion
0	2.33±0.39	–	–
24	–	$1.77\pm0.26^{+}$	1.45±0.26
72	–	$1.78\pm0.32^{+}$	1.82±0.23

$^{+}$ indicates significant difference ($p<0.05$) between the control and GalN group.

Discussion

In the present study, we examined the effect of G-galactosamine, a hepatotoxic agent, on the liver and the capability of specific nutritional supply to influence liver damage. We used triacylglycerols containing medium chain fatty acids (lipid emulsion Lipofundin MCT/LCT 10% / Miglyol[812]) because these fatty acids may cross the mitochondrial membrane independently of the carnitine carrier system and are thus better available for tissue oxidation.

We verified that GalN administration induced liver injury as indicated by an increase of serum aminotransferase activities. We observed the decrease of total liver proteins evidently due to liver injury and also significant increase of the content of triacylglycerols (Tables 1 - 3). When comparing the effect of GalN alone with the effect of GalN and lipid emulsion applied for 24, 48, and 72 h, we found that lipid emulsion participates in

improving the conditions for liver reparation. The combination of GalN and lipid emulsion attenuated the decrease in the total liver protein content during the first 48 h compared with the GalN group. We cannot exactly explain why this protective effect of lipids was not evident at 72 h after GalN administration. It could be due to a negative effect of steatosis induced by lipid emulsion administration (Table 3). A similar finding was reported by Mangeney *et al.* (1985) in isolated rat hepatocytes. Their data indicated that GalN induces a decrease of triacylglycerol secretion and inhibits protein synthesis and secretion.

Lipid emulsion-treated animals have a higher total content and specific activity of cytochrome c oxidase 24, 48, and 72 h after GalN administration. In the group with GalN, we found a smaller increase of enzyme content and activity at 48-h and 72-h intervals when the regeneration process started. In the group with lipid emulsion, the increase in both content and activity of cytochrome c oxidase was already found at the 24-h interval. The decrease of total liver protein accompanying higher activity of cytochrome c oxidase suggests earlier reparation of respiratory chain enzymes compared with other cell proteins. Furthermore, cytochrome c oxidase indicates the capacity of the whole mitochondrial system of energy production. Our data suggest that mitochondrial biogenesis was activated after GalN administration because mitochondria isolated from liver of GalN and GalN plus lipid emulsion-treated groups have the same activity of cytochrome c oxidase (Table 5). A similar protective effect of the lipid emulsion on cytochrome c oxidase activity in liver homogenate was observed when liver injury was induced by thioacetamide (Červinková and Drahota 1998). In comparison with control rats the mitochondrial cytochrome c oxidase activity in GalN and GalN + lipid emulsion groups was lower (Table 5). We cannot specify from our experimental data whether this was due to the toxic effect of GalN or due to biogenesis of mitochondria with lower specific cytochrome c oxidase activity.

We may conclude from our experiments that lipid emulsion (Lipofundin MCT/LCT 10 % / Miglyol[812]) significantly diminished the extent of liver injury induced by GalN and contributed to improvement of conditions for restoration of liver tissue and its function in the following regeneration process. Lipid supplementation may thus be an important factor supporting optimum metabolic conditions for the high rate of energy-dependent recovery processes repairing the liver tissue damaged by GalN intoxication.

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Fatty Acid Profile and Oil Yield in Six Different Varieties of Fresh and Dry Samples of Coconuts (*Cocos nucifera*)

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Abstract: The physical and chemical properties of the oil extracted from six (6) varieties of coconut fruits were determined. The oils were brownish, some yellowish when melted, and at times white when solidified. The oils had specific gravity ranging between 0.90 and 0.92. The yield of the oil (% contents) from the ether extract was also analyzed using soxhlet extraction technique. The results showed the oil contents of 88.54% in dry samples and 69.14% in fresh samples. Result of the findings showed a higher percentage of saturated fatty acid content across the entire samples (88.5±0.85% - 97.0±0.37%) in the dry samples, than the unsaturated fatty acid constituents (8.4±0.45% - 9.5±0.30%). The moisture content was higher in the fresh samples than the dry samples. These findings therefore suggest a high percentage oil yield in the dry samples. It is therefore advisable that the nuts be harvested dry if the oil is intended for commercial use.

Key words: Oil yield, fatty acid, cocos nucifera

Introduction

The natural fats and oil are mixtures of glycerides of fatty acids. Fats and oil are naturally occurring organic compounds which belong to a large group of water insoluble substances called lipids. They are insoluble in water and soluble in organic solvents such as ether, chloroform, benzene, turpentine (Anosike, 1994).

World supplies of fats and oil are reported to come from vegetable sources (68.1%), animal fat (28.2%), marine fat (3.8%) (Fox and Cameron, 1984). In Nigeria, there are abundant sources of lipids such as palm oil, coconut oil, groundnut oil, rubber seed oil, cotton seed oil, soya bean oil, camphor seed oil etc.

Lipids are sometimes classified based on their degree of unsaturation. For example, arachidonic acid and linolenic acid. Those ones classified based on the degree of unsaturation include lauric, stearic, myristic palmitic acids etc. (Borgstrom, 1968).

The coconut has been a traditional food in practically all the countries where it is grown, and the quantity of fresh coconuts consumed locally varies from over 90% of the total population, in Thailand to less than 2% in the Philippines (Dendy, 1984).

Coconut play important role in diets in many ways: the tender nut is used for their water, mature nut for cooking and the preparation of sweetmeats, and oil for home consumption. Probably the best known product is coconut milk, the oil-protein-water emulsion obtained when the grated fresh coconut meal (endosperm) is squeezed through a muslin cloth (Dendy, 1984). Ripe or fully mature coconut (ball copra or cup copra) is used in religious and sacrificial offering, occupying a very important place in Hindu.

Through experimental studies on lipids from different oil seeds, the component of these lipids have been obtained. Also efforts have been made to improve both their nutritive value and their utilization for the production of materials for cosmetics: creams, lotions, perfumes, lipstick, eye-shadow, face cream for rapid economic utilization of the oil (Braverman, 1963).

Materials and Methods

Collection and Preparation of Samples before Analysis: A hybrid of coconut variety, PB121 (MYD X WAT) was purchased at NIFOR (National Institute for Oil Palm Research) Benin, Edo State, Nigeria in June, 2002.

Four other samples of different varieties were collected from Efa In Etinan Local Government Area and one varieties from Obot Idim Ibesikpo, Ibesikpo-Asutan Local Government Area, Akwa Ibom State, Nigeria in June, 2002.

Description of the Samples: Eight different varieties of coconut were obtained in its fresh (immature) and dry (fully ripe) conditions.

i) Chowgat Dwarf Green (CDG) (fresh and dry samples). It is characterized by its small size, green husk and short height. Locally known as "Nda Kisong".
ii) Hybrid coconut PB121 obtained by crossing Malayan Yellow Dwarf X West African Tall (fresh and dry samples). It is characterized by its larger size than CDG short and fat stem. It may bear as much as 15-20 nuts per bunch.
iii) West Africa Tall Green (fresh and dry samples). This is most commonly seen in our locality and is marked by its tall trunk and medium sized nuts. It bears fruit

with green colour which turns brown at maturity.

iv) Chowgat Dwarf Orange (fresh and dry samples). This is not widely used for oil production but rather as an ornament because of its bright colour, it is characterized by its thin stem, orange colour nut and fronds.

v) Lakshadweep Ordinary (fresh and dry samples). It has a medium sized trunk and is characterized by its three lines on the husk and has a large brown husk at its later stage and finally turns brown on maturity.

vi) Adaman Giant (fresh and dry samples). Locally know as "Abude". It is medium in height and its name is originated from its exceedingly large nut. It bears 5-9 fruits per bunch. It has a green shell at the early stage and then turns brown at maturity.

Preparation of samples for analysis: The samples were dehusked, labelled and then taken to University of Jos, Nigeria where the analysis took place.

A simple cutter was used to slit each raw coconut open and a knife was used immediately to remove the nut from the hard shell. A portion of the fruit was divided for the determination of the moisture content from each variety. A similar portion from each variety was dried in Gallenkamp air oven at 40-60^{0}C for an oil extraction. The remaining parts were stored in both an oven and fridge for further use.

Analysis of sample: The determination of the moisture content was carried out using AOAC (1975).

Extraction of the Oil from the Kernel: A portion of the oven-dried kernel (copra) was ground into fine particles with a manual grinder. 10g of each of the samples was packed into a weighed filter paper and wrapped with thread. This was then dropped into soxlet apparatus and the oil extracted using petroleum ether (Boiling point 40-60°C).

Determination of Oil Content of the Ether Extract (AOAC, 1975): Ten gram of each of the ground sample was obtained for extraction of oil in apparatus. The oil content in each sample was obtained and the output recorded as the ratio of the weight of the petroleum ether extracted oil to the weight of the sample.

The oil extract was then poured into the round bottom flask of the distillation apparatus, which was fitted to a condenser and clamped at two positions. After the distillation, the oil was obtained in a flask while the petroleum ether was collected with a beaker as it dripped from the condenser.

Determination of the specific gravity of the oil (relative density – British Standard Method of Analysis, 1958): The specific gravity of the oil was determined using density bottle. Some portions of the oil sample in each variety and water was weighed separately at room temperature. The specific gravity was obtained and reported as the ratio of the mass of the oil used to the mass of the water at room temperature.

An aliquot of each of the oil samples was taken into a capillary tube. The capillary tube together with thermometer were inserted into a melting point apparatus. The apparatus was then switched on and the temperature at which the oil melted was recorded. This was repeated for two times to obtain a constant reading in each of the samples.

The temperature at which the oil begins to set was determined by putting some oil in a sterile container and then placed in the refrigerator and the thermometer was also inserted to record the setting temperature.

Determination of fatty acid profile: Detection of Vegetable Fat in each coconut oil based on the presence of saturated and unsaturated Fatty Acids by gas liquid chromatography (BSI, 1958) 50mg of melted fat from each sample was taken in a glass stoppered test tube for the preparation of fatty acid methyl ester. The methyl ester were extracted with petroleum ether (40-60°C) and concentrated under nitrogen before injecting to gas chromatograph. The column temperature was 185°C. The flow rate of carrier gas nitrogen was maintained at 2.8kg/cm^2 (25ml/mins) and chart speed at 1cm/min.

The peaks of concentration in each case with the standard and the unknown samples were matched. The percentage fatty acid constituents were obtained by the display unit of the instrument.

Results and Discussion

The colour of the oil in each sample ranges from yellow to brown at room temperature. The oil was soluble in chloroform and petroleum ether, this solubility was useful in the extraction and isolation of the oil. The setting temperature of all the samples ranged between 16.00 ± 0.01 to 19.40 ± 0.21 being higher than that of unripe paw-paw seed oil of 8.00±0.02°C. The oil also has a specific gravity of between 0.90 to 0.92. This is comparable to that of the early workers (Johnson and Peterson, 1974; Solly and Dass, 1980; Borgstrom, 1968).

The melting point of the oil ranged between 25°C to 26°C for all the samples. This range is generally lower than the melting point of Butter (36°C), Palm oil (39°C) Palm Kernel oil (29°C), Lard (43°C), (Fox and Cameron, 1984).

The moisture content is seen to be higher in fresh samples than the fully ripe ones. The moisture content which is often used as an index of stability and quality as well as a measure of yield and quantity of solid food is generally lower for dry nuts. This is suitable compared with the result obtained by various workers (45-46,76) in fully ripe nuts (Johnson, 1987).

Table 1: Result and some physical properties of different varieties of coconut

Varieties of coconut	Sample Type	Specific Gravity (SG)	Setting Point (SP)	Moisture Content %	Oil Yield (%)
Chowgat Dwarf Green (CDG)	Fresh	ND	ND	55.61	49.25
	Dry	0.90	18.0±0.02	48.25	57.61
Hybrid PB121	Fresh	ND	ND	51.83	49.34
	Dry	0.91	16.8±0.01	46.59	65.15
West African Tall Green	Fresh	ND	ND	57.22	69.14
	Dry	0.91	19.4±0.21	44.55	71.04
Chowgat Dwarf Orange	Fresh	ND	ND	61.34	45.26
	Dry	0.92	16.0±0.01	44.71	66.59
Lakshadweep Ordinary	Fresh	ND	ND	40.27	50.83
	Dry	0.91	18.5±0.01	40.01	78.71
Adaman Giant	Fresh	ND	ND	58.70	55.51
	Dry	0.91	18.5±0.01	32.40	88.54

ND - Not Detected

Table 2: Fatty acid profile of different varieties of coconut

Variety of coconut	Sample Type	Caproic	Caprylic	Capric	Lauric	Myristic	Palmitic	Stearic	Total Saturated
Saturated									
Chowgat	Fresh	0.10±0.01	4.60±0.12	4.00±0.11	43.00±0.11	13.10±0.01	5.40±0.02	0.10±0.01	70.55±0.30
Dwarf Green	Dry	0.30±0.02	5.6±0.01	9.50±0.02	45.00±0.01	17.00±0.01	10.50±0.21	3.40±0.14	91.30±0.43
Hybrid	Fresh	0.10±0.01	4.50±0.02	4.30±0.13	40.00±0.13	14.00±0.03	5.5±0.10	0.10±0.01	73.30±0.41
PB121	Dry	0.40±0.01	5.60±0.01	9.50±0.02	40.00±0.13	13.5±0.13	10.0±0.32	3.00±0.02	83.5±0.85
West African	Fresh	0.20±0.01	8.50±0.02	9.00±0.01	40.00±0.01	12.50±0.01	10.00±0.10	2.50±0.10	84.20±0.37
Tall Green	Dry	0.50±0.01	9.00±0.02	9.50±0.01	45.00±0.20	9.50±0.01	10.50±0.01	2.50±0.10	88.00±0.37
Chowgat	Fresh	0.10±0.01	4.60±0.01	4.00±0.02	44.00±0.11	15.00±0.01	6.00±0.01	2.50±0.02	76.40±0.20
Dwarf Orange	Dry	0.50±0.01	5.5±0.02	9.50±0.02	40.00±0.01	16.00±0.01	10.50±0.14	3.40±0.14	86.40±0.35
Lakshadweed	Fresh	0.10±0.01	5.40±0.01	4.70±0.01	44.00±0.11	14.00±0.12	10.40±0.11	2.00±0.20	80.60±0.37
Ordinary	Dry	0.40±0.01	10.0±0.01	9.50±0.01	38.00±0.02	18.00±0.01	10.0±0.01	3.10±0.01	90.50±0.09
Adaman Giant	Fresh	0.20±0.01	5.40±0.10	4.70±0.10	43.00±0.10	15.50±0.03	10.50±0.40	2.50±0.01	82.70±0.76
	Dry	0.40±0.01	9.00±0.01	7.00±0.01	40.60±0.01	16.50±0.20	10.50±0.01	3.50±0.01	89.50±0.27

Variety of coconut	Sample Type	Arachidonic	Oleic	Linoleic	Total Unsaturated
Unsaturated					
Chowgat	Fresh	0.25±0.01	7.50±0.02	2.90±0.21	10.40±0.23
Dwarf Green	Dry	1.00±0.01	4.9±0.23	2.20±0.01	7.4±0.24
Hybrid	Fresh	0.30±0.01	16.0±0.31	2.00±0.14	18.00±0.45
PB121	Dry	1.50±0.21	8.00±0.20	1.50±0.10	9.50±0.30
West African	Fresh	1.50±0.01	6.50±0.01	2.50±0.02	9.00±0.03
Tall Green	Dry	1.50±0.01	5.50±0.02	1.50±0.01	7.00±0.03
Chowgat	Fresh	0.20±0.01	7.50±0.20	3.00±0.10	10.50±0.30
Dwarf Orange	Dry	1.00±0.10	4.50±0.01	2.60±0.01	7.10±0.11
Lakshadweed	Fresh	1.00±0.00	6.80±0.10	1.40±0.11	8.20±0.21
Ordinary	Fresh	1.50±0.01	7.50±0.02	1.60±0.00	9.10±0.01
Adaman Giant	Fresh	0.90±0.01	9.00±0.02	1.30±0.01	10.30±0.03
	Dry	2.00±0.01	6.40±0.01	2.60±0.03	9.00±0.04

The oil content (crude fat) of oil seeds is of great significance in the determination of the value of the produce for processing. For the kernel, the value is significantly high and cuts across the different varieties considered and also in the fresh and dry samples. These range from the highest value of 88.5% in Andaman Giant to the least of 57.61% in the Chowgat Dwarf Green. The percentage oil yield, in all samples ranged between 57.61 to 88.54%. This is higher than that of groundnut (49.90%), sesame seed (46.90%), and palm kernel (49.00%) (Fox and Cameron, 1984).

The oil yield in dry sample was significantly higher than fresh sample. The highest value in dry sample was 88.54% and in fresh 69.14%. These samples showed a close range with that of Johnson (1987) with fresh having 68.20±0.10% and dry 85.00±0.10%. The values are higher than those of rubber seed oil 23.96% and that of groundnut oil 50.00%.

The characteristic fatty acid constituent in the coconut oil of each variety (Table 2) show no significant difference from those of the earlier workers. The total saturated fatty acid is seen to be much higher than the unsaturated

fats. The total saturated fatty acids ranged between 88.50±0.85% to 97.00± 0.37% in the dry samples. This conforms with 91.00% (Noller, 1965) and 92.00% (Johnson and Peterson, 1974) reported in coconut. The unsaturated fatty acids ranged from 8.40±0.45% to 9.50±0.30% in dry samples. This conforms with 9.00 to 9.10% (Noller, 1965; Johnson and Peterson, 1974) for dry coconut samples.

There is also a slight increase in the unsaturated fatty acid values in the fresh samples. These values decrease gradually as the nut become fully mature which accumulate greater percentage of saturated fatty acids.

Table 2 also show Lauric acid (40.00 to 50.00%) and Myristic acid (13.50 to 18.50%) as the major saturated fatty acids present in coconut oil. This compare with those reported for lauric acid (47.60%) and Myristic acid (15.80%), (Tyler *et al.*, 1977). There are also a mono unsaturated fatty acids: Oleic acid (5.90 to 8.00%) and Linoleic (1.50 to 2.60%).

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